WEST Search History

DATE: Monday, April 01, 2002

Set Name side by side	Query	Hit Count	Set Name result set
	DB=USPT; PLUR=YES; OP=AND		
L1	5972704.pn. and 201	1	L1
L2	6165788.pn. and 19	1	L2
L3	5976795.pn. and 20	1	L3
L4	5693535.pn. and 201	1	L4
L5	promoter.clm.	9751	L5
L6	L5 same transcri$.clm.	1360	L6
L7	L6 and clostrid$.clm.	2	L7
L8	clostrid$ near25 (transci$ or promot$)	35	L8
L9	L8 notl7	0	L9
L10	L8 not l7	34	L10

END OF SEARCH HISTORY

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DATE: Monday, April 01, 2002

Set Name side by side	Query	Hit Count	Set Name result set
	DB=USPT; PLUR=YES; OP=AND		
L1	5874220.pn. and (promot$ or transcri$)	1	L1
L2	l1 and initi$	1	L2
L3	L2 and ribosom$	0	L3

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L10: Entry 14 of 34 File: USPT Sep 21, 1999

DOCUMENT-IDENTIFIER: US 5955368 A
TITLE: Expression system for clostridium species

Abstract Paragraph Left (1):
A system is used to express clostridial gene constructions in a clostridial host. A mobilizable transfer plasmid is described which permits the direct transfer of the plasmid, and genes carried on it, from E. coli into Clostridium species. A promoter is described for use in clostridial species. Also, a useful host strain is used which is nontoxigenic and which permits high levels of expression of clostridial genes using the clostridial promoter.

Brief Summary Paragraph Right (13):
The present invention is summarized in that an expression system has been developed for the expression of clostridial genes in a toxin deficient clostridial strain which makes use of a broad range host plasmid which is capable of promoting conjugative transfer of genes from E. coli to clostridial species.

Brief Summary Paragraph Right (14):
It is an attribute of the clostridial gene expression system of the present invention in that it has been found that the promoter region of the nontoxic nonhemagglutinin (NTNH) gene is a particularly efficient gene expression driver in clostridial species, and, in particular, functions as an highly effective gene expression driver in strains which are toxin deficient.

Brief Summary Paragraph Right (15):
The present invention is further novel in that the combination of a host strain, the shuttle plasmid, and the particular promoter, enables the high expression of any clostridial gene toxins or toxin fragments in clostridial species thereby taking advantageous use of the inherently preferential pattern of gene expression of these species for genes of species in the same genus.

Detailed Description Paragraph Right (1):
Described herein are a series of components which, when taken together, make up a system which permits the efficient delivery of heterologous genes into a clostridial host, such as C. botulinum, and the expression of genes in that host. One element of this system includes a strain of C. botulinum which does not produce a toxin prior to transformation or conjugation, and therefore does not make interfering or contaminating toxin fragments or toxins which might hinder the purification or processing of heterologous toxins made in the C. botulinum strain. Also described is a clostridial promoter system which is capable of expressing genes in clostridial species and which turns out to be highly efficiently expressed in the toxin deficient C. botulinum strain used in the examples described below. Also disclosed is a shuttle vector plasmid which is capable of being constructed in commonly used bacteria, such as E. coli, and which may then be transferred by

conjugation into clostridial species. Taken together, these components provide a system which permits the efficient construction of genes for expression in clostridial species, a system for the convenient transfer of genes into clostridial species, and the mechanism to ensure abundant expression of those genes once inserted into a clostridial host. Thus it becomes possible to make any number of clostridial toxins, toxin fragments, or antigenic portions thereof, in a clostridial host in a way both that ensures abundant expression and facilitates purification. Furthermore, toxins with altered structures, chimeric toxins, and other toxin derivatives valuable in medicine could be synthesized in this system.

Detailed Description Paragraph Right (7):
Obviously, to prepare a genetic construct for use in Clostridium species, it is necessary to use a promoter effective in these species. Illustrated in FIG. 2 is the origin of the promoter elements used in the practice of the invention described below. On the top panel of the figure is illustrated the arrangement of gene components in the native botulinum toxin gene cluster. Open reading frames are indicated as arrows showing the orientation of transcription. The designation BoNT refers to the botulinum neurotoxin gene, while the designation NTNH refers to the nontoxic non-hemagglutinin gene and HA refers to the hemagglutinin genes. The designation ORFX refers to open reading frame X. The lower panel of FIG. 2 illustrates the components from the C. botulinum genome that were used to construct the hybrid NTNH-LC gene. This gene contains the ORFX region combined with a promoter and an amino terminal encoding region from the NTNH gene driving the expression of the light chain portion of the BONT toxin, designated in FIG. 2 as "LC". It is demonstrated in the examples described below that the promoter from NTNH is contained within this fragment and is capable of driving downstream fragments to express in C. botulinum. It is believed, but not demonstrated, that the amino terminus of the NTNH protein coding region assists in the efficient expression of the chimeric hybrid protein molecule in this host.

CLAIMS:

6. A method for the delivery and expression of genetic constructs in a Clostridium species comprising the steps of:

making a genetic construction including a promoter effective in the Clostridium species;

inserting the genetic construction in a mobilizable transfer plasmid which includes an origin of replication effective in E. coli; an origin of replication effective in a Clostridium species; a gene for an antibiotic resistance marker; and an origin of conjugative transfer which, when actuated, is capable of directing the transfer of the plasmid from E. coli into a Clostridium species;

transforming the mobilizable plasmid into an E. coli strain;

culturing the E. coli strain carrying the plasmid with a culture of the Clostridium species under conditions which facilitate conjugative transfer of the plasmid; and

selecting for bacteria of the Clostridium species which are hosting the plasmid.

8. A method as claimed in claim 6 wherein the promoter effective in Clostridium species is the NTNH promoter from Clostridium botulinum.

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L10: Entry 19 of 34 File: USPT Jun 2, 1998

DOCUMENT-IDENTIFIER: US 5759845 A
TITLE: Secretion of clostridium cellulase by E. coli

Brief Summary Paragraph Right (8):
Preferably, the expression vector of the transformed microorganism provided by the present invention comprises a first DNA sequence coding for a cellulase obtained from a Clostridium strain IY-2 and a second DNA sequence coding for a Clostridium promoter. Thus, the E. coli host is capable of synthesizing and secreting extracellular cellulase at 28.degree. C. The expression of the cellulase at 28.degree. C. suggests that the promoter for that gene or some other promoter-like sequences that can be recognized by E. coli RNA polymerase have also been cloned with the cellulase structural gene. The results further suggests that E. coli RNA polymerase can utilize the cellulase gene's promoter to initiate transcription and subsequent expression of the Clostridium cellulase in E. coli.

Brief Summary Paragraph Right (9):
The expression plasmid of pLC2833, 2.8 kb DNA fragment which contains the cloned cellulase gene from Clostridium, is further subcloned by restriction enzyme digestion into a second plasmid containing a 2.2 kb DNA insert. The referenced 2.2 kb DNA fragment contains at least the entire structural gene of cellulase and its promoter from Clostridium strain IY-2. The plasmid which contains the 2.2 kb Clostridium DNA fragment is named pPCIA. Any microorganism which is known in the art to be amenable to the DNA mediated transformation is conceivably a target host for the present invention. A preferred host microorganism provided by the present invention is an Escherichia bacterium. Preferably, the microorganism host is E. coli K-12.

Brief Summary Paragraph Right (11):
The present invention further provides a method for the digestion of untreated natural plant materials. One generalized method comprises incubating untreated or pretreated natural plant materials with the novel cellulose enzyme of the present invention and recovering the products produced therefrom. A specific method comprises: (a) transforming a host microorganism with a vector wherein said vector containing one or more DNA sequences coding for one or more gene products, signal sequences, or regulatory sequences useful in the production and secretion of cellulase; (b) growing said transformed bacterium in a suitable growth medium; and (c) incubating the untreated natural plant materials with said growth medium. Preferably, the transformed microorganism is a Escherichia bacterium containing DNA sequences code for cellulase and its promoter obtained from a Clostridium strain IY-2. A preferred transformed strain is E. coli K-12. The untreated natural plant materials are selected from the group consisting of bamboo fibers, cellulose, rice straw or any other post-harvest agricultural plant waste. The products produced from these untreated natural plant materials consist of cellobiose, cellotetraose and larger soluble polymers of cellulose, which can be used as fermentation feed stocks for producing a variety of useful products such as ethanol, the latter of

which can be produced by known or standard fermentation conditions.

Detailed Description Paragraph Right (27):
The regulated high level E. coli expression vector pLC2833 (Remaut et al. Gene 15:81-93 (1981)) containing a .lambda.P.sub.L promoter was initially chosen for the isolation of the Clostridium cellulase genes, because continuous high level production of cellulase may be detrimental to the host-vector system. The transcription from .lambda.P.sub.L promoter is repressed at low temperatures and derepressed after shifting to 42.degree. C. for one hour. The recombinant plasmids containing partially Sau3A digested Clostridium DNA fragments were transformed into E. coli K-12 and cellulase producing clones were isolated as described above. The results also revealed that cellulase activity was found whether the culture was grown at 28.degree. C. or at 42.degree. C. (Table 2). Thus the activation of the .lambda.P.sub.L promoter was not necessary for the expression of the cellulase gene.

CLAIMS:

9. The method of claim 8 in which the expression vector contains a lambda P.sub.L promoter and a 2.8 kb Clostridium strain IY-2 DNA insert.

L10: Entry 24 of 34 File: USPT Mar 5, 1996

DOCUMENT-IDENTIFIER: US 5496725 A
TITLE: Secretion of Clostridium cellulase by E. coli

Brief Summary Paragraph Right (9):
Preferably, the expression vector of the transformed microorganism provided by the present invention comprises a first DNA sequence coding for a cellulase obtained from a Clostridium strain IY-2 and a second DNA sequence coding for a Clostridium promoter. Thus, the E. coli host is capable of synthesizing and secreting extracellular cellulase at 28.degree. C. The expression of the cellulase at 28.degree. C. suggests that the promoter for that gene or some other promoter-like sequences that can be recognized by E. coli RNA polymerase have also been cloned with the cellulase structural gene. The results further suggests that E. coli RNA polymerase can utilize the cellulase gene's promoter to initiate transcription and subsequent expression of the clostridium cellulase in E. coli.

Brief Summary Paragraph Right (10):
The expression plasmid of pLC2833, 2.8 kb DNA fragment which contains the cloned cellulase gene from Clostridium, is further subcloned by restriction enzyme digestion into a second plasmid containing a 2.2 kb DNA insert. The referenced 2.2 kb DNA fragment contains at least the entire structural gene of cellulase and its promoter from Clostridium strain IY-2. The plasmid which contains the 2.2 kb Clostridium DNA fragment is named pPC1A. Any microorganism which is known in the art to be amenable to the DNA mediated transformation is conceivably a target host for the present invention. A preferred host microorganism provided by the present invention is an Escherichia bacterium. Preferably, the microorganism host is E. coli K-12.

Brief Summary Paragraph Right (12):
The present invention further provides a method for the digestion of untreated natural plant materials. One generalized method comprises incubating untreated or pretreated natural plant materials with the novel cellulase enzyme of the present invention and recovering the products produced therefrom. A specific method comprises: (a) transforming a host microorganism with a vector wherein said vector containing one or more DNA sequences coding for one or more gene products, signal sequences, or regulatory sequences useful in the production and secretion of cellulase; (b) growing said transformed bacterium in a suitable growth medium; and (c) incubating the untreated natural plant materials with said growth medium. Preferably, the transformed microorganism is a Escherichia bacterium containing DNA sequences code for cellulase and its promoter obtained from a Clostridium strain IY-2. A preferred transformed strain is E. coli K-12. The untreated natural plant materials are selected from the group consisting of bamboo fibers, cellulose, rice straw or any other post-harvest agricultural plant waste. The products produced from these untreated natural plant materials consist of cellobiose, cellotetraose and larger soluble polymers of cellulose, which can be used as fermentation feed stocks for producing a variety of useful products such as ethanol, the latter of

which can be produced by known or standard fermentation conditions.

Detailed Description Paragraph Right (24):
The regulated high level E. coli expression vector pLC2833 (Remaut et al. Gene 15:81-93 (1981)) containing a .lambda.P.sub.L promoter was initially chosen for the isolation of the Clostridium cellulase genes, because continuous high level production of cellulase may be detrimental to the host-vector system. The transcription from .lambda.P.sub.L promoter is repressed at low temperatures and derepressed after shifting to 42.degree. C. for one hour. The recombinant plasmids containing partially Sau3A digested Clostridium DNA fragments were transformed into E. coli K-12 and cellulase producing clones were isolated as described above. The results also revealed that cellulase activity was found whether the culture was grown at 28.degree. C. or at 42.degree. C. (Table 2). Thus the activation of the .lambda.P.sub.L promoter was not necessary for the expression of the cellulase gene.

CLAIMS:

2. A microorganism of claim 1 wherein the expression vector is an E. coli plasmid, pLC2833, containing a .lambda.P.sub.L promoter and a 2.8 kb Clostridiim strain IY-2 DNA insert.

3. A microorganism of claim 2 wherein said 2.8 kb insert is further cleaved into 2.2 kb DNA fragment comprising at least an entire structural gene and signal sequences of cellulase and the promoter is from the Clostridium cellulase gene on plasmid pPC1A.